

# Microbial Terroir: Isolation of Natural Yeasts from Vineyard/Winery Operations for Use in Wine Production

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Cool  
Climate  
Oenology &  
Viticulture  
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Brock University

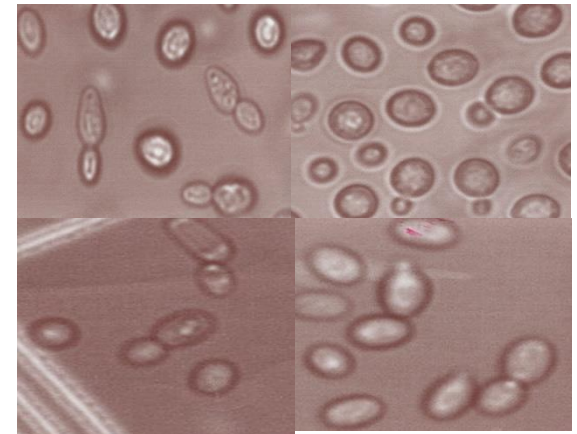
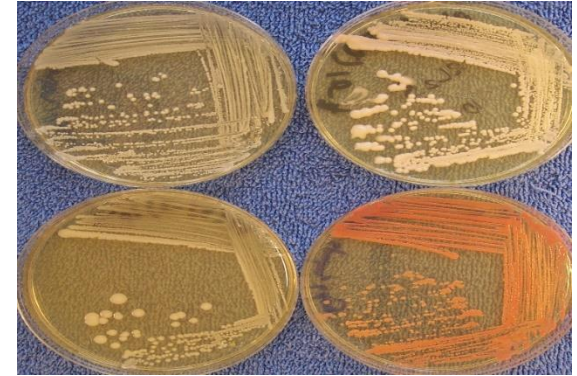




# Microbial Terroir



- Growing interest worldwide, including Canada, to further differentiate wines by expressing a region's microbial terroir
  - Use the natural yeast on the fruit to ferment wine
  - Add complexity to wine due to contributions of many yeast species
  - Hall et al, 2011; Jolly et al, 2014; Scholl et al, 2016; Morgan et al, 2019; Kelly et al, 2020; McCarthy et al, 2021; Bunbury-Blanchette et al, 2022
- **Risks: inconsistency, off flavours, oxidation faults**



# Commercial *S. cerevisiae* offer control over a fermentation, but is regional identity sacrificed?



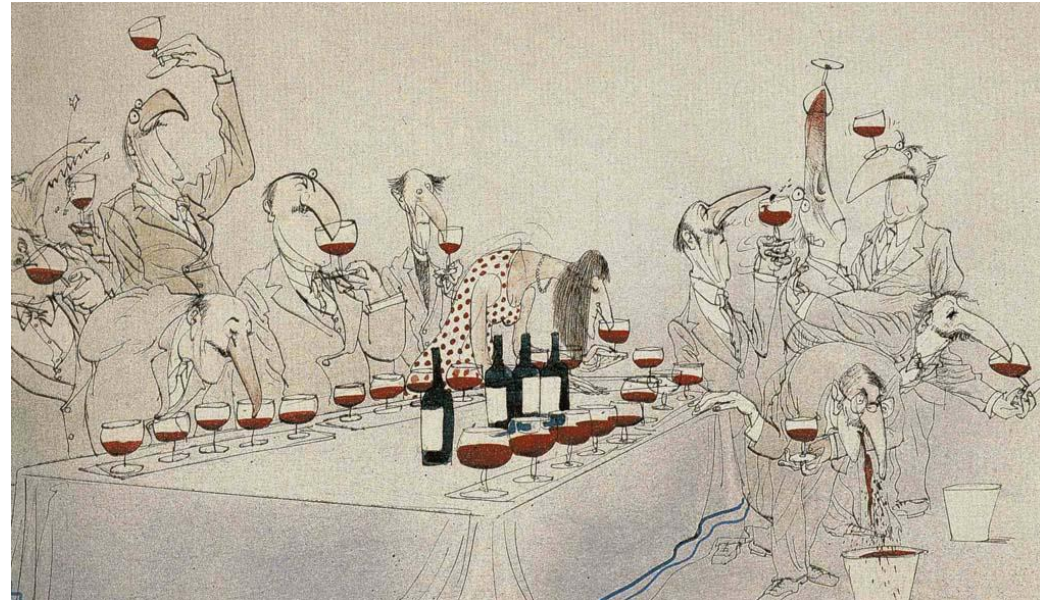
- Most commercial yeast on the market are of the species *Saccharomyces cerevisiae* although a handful of non-*Saccharomyces* yeast are entering the market
  - *Saccharomyces cerevisiae*:
    - alcohol tolerant
    - can complete fermentation
    - selected for oenological performance and flavour profile of resultant wines
- Most *S. cerevisiae* were selected from European countries, may not be the best performing yeast for our cool climate regional wines

# Commercial Yeast versus Spontaneous Fermentation



## Commercial yeast

- Wines may not exhibit characteristics of the local microflora but there is more control over the ferments, less risk of stuck fermentations and off flavours due to yeast strain



## Spontaneous Fermentation

- Some winemakers like the complexity of working with indigenous, natural yeast but not all indigenous yeast can complete a fermentation, and many can produce off flavours
- More difficult to control a natural, spontaneous fermentation because you are not sure what yeast will be present

# Can winemakers differentiate their wines with their own yeast but still attain consistency and quality?



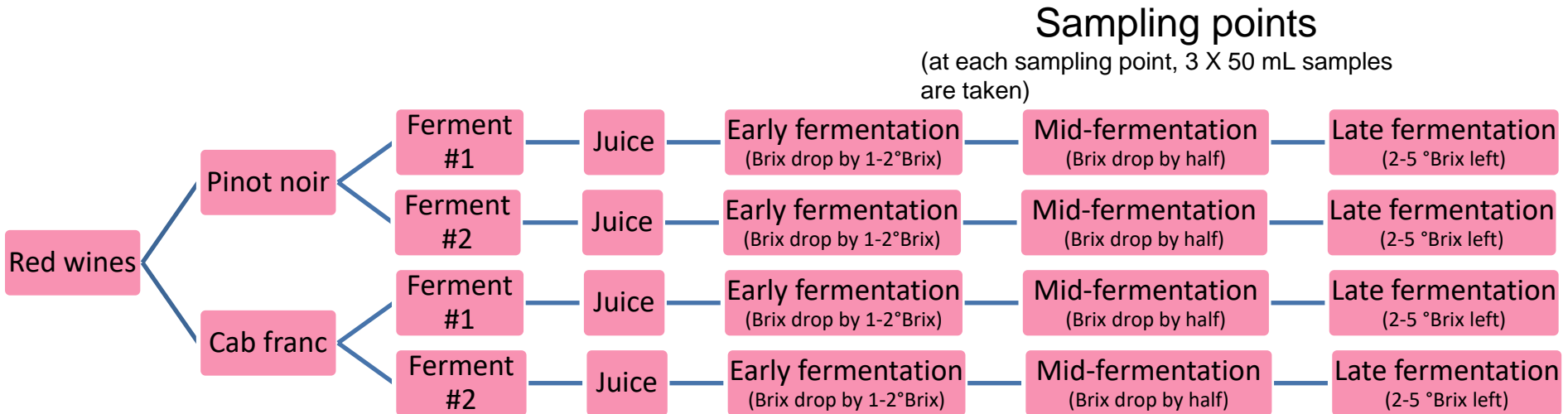
- YES, we have the tools to isolate your own yeast
  1. Select yeast from a spontaneous fermentation, focus on isolates that are able to complete a fermentation
  2. Identify the yeast isolates to species, then strain level
  3. Trial isolates from culture preparations in small scale ferments to select best performing yeast
  4. Utilize a local yeast company to grow up the cultures in paste form for commercial fermentation



# Step 1. Spontaneous fermentation

## Trial with local winery,

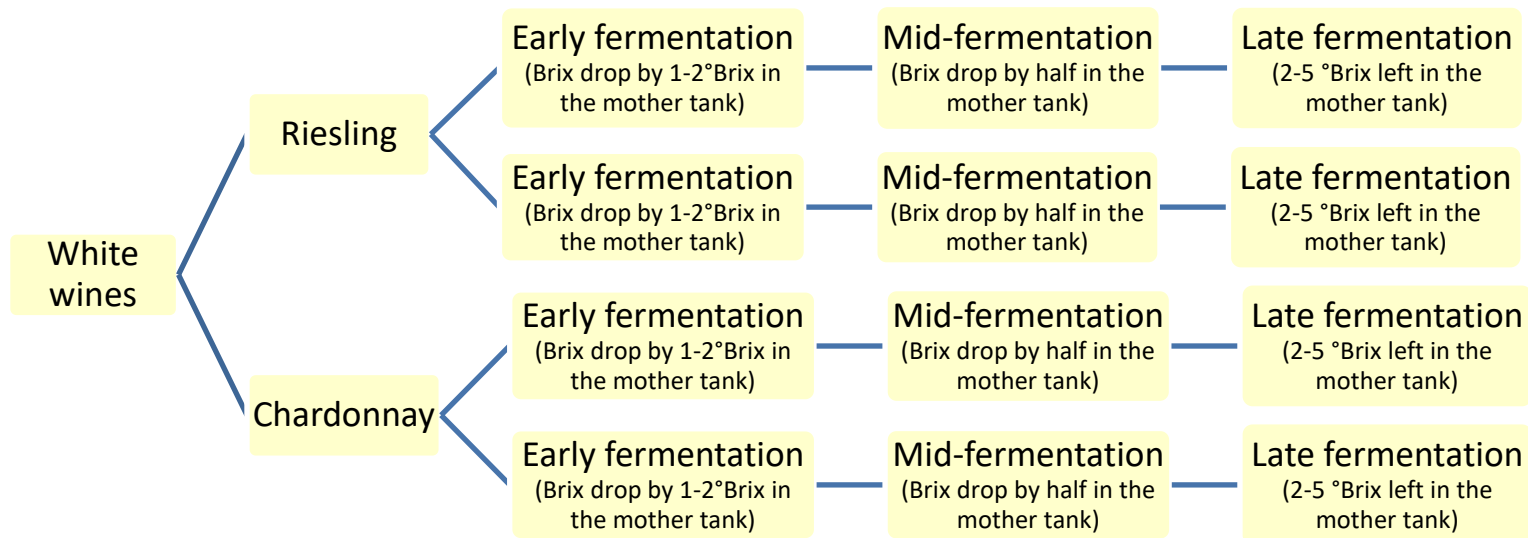
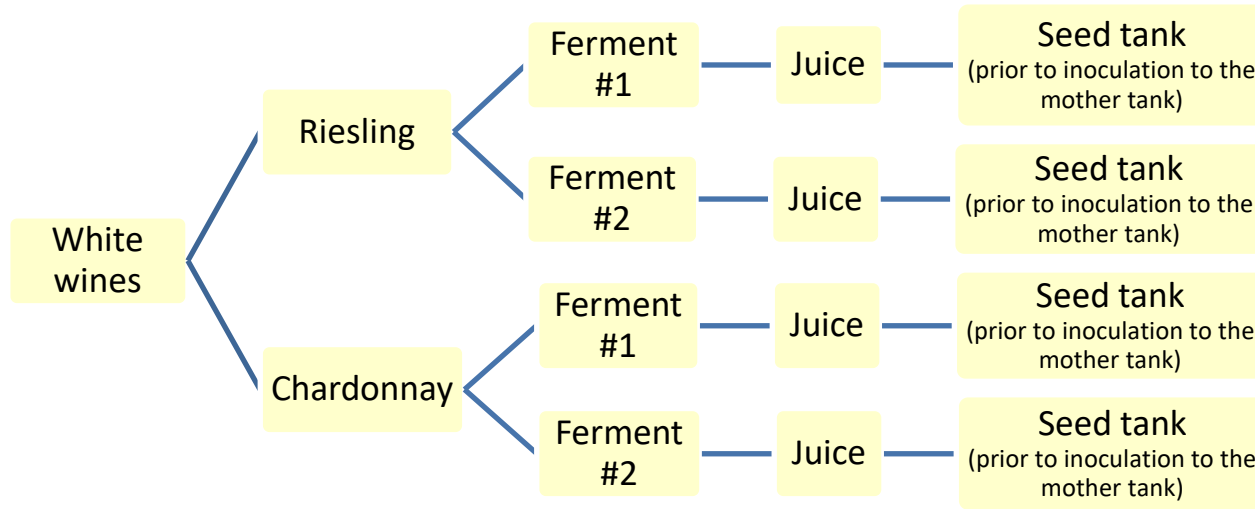
### Experimental design: Red wine



- Ferments were performed in duplicate at the winery
- Sampled at 3 different stages of the ferment:
  - early, middle and end (triplicate sampling at each timepoint, 3 X 50 mL)
- Only identified and selected yeast from end stages of fermentation

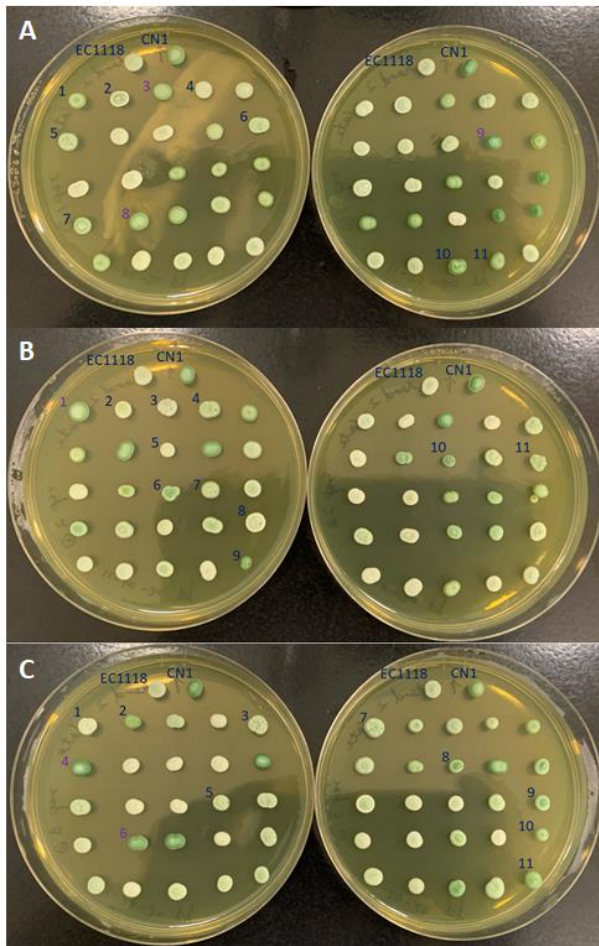
# Trial with local winery

## Experimental Design: white wine



# Step 2. Yeast isolation and identification

## Chardonnay, late stage sampling



**Figure 1. Results of preliminary identification of colonies isolated from the 2<sup>nd</sup> Chardonnay fermentation**

**-Green colonies *S. uvarum***

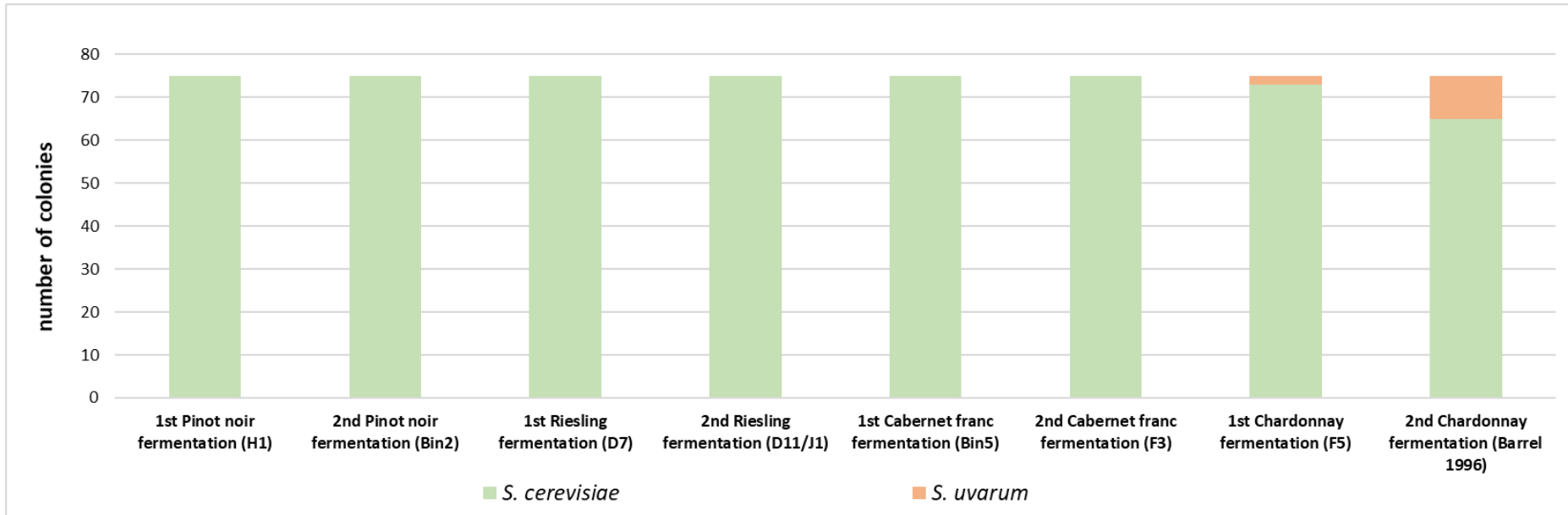
**-Cream colonies *S. cerevisiae***



-25 isolates were selected from each YPD plate, replated on WLN differential media for preliminary identification as *S. cerevisiae* or *uvarum*

-Yeast identification confirmed using molecular techniques (PCR)



# Comparison of yeast isolates across all fermentations



- 600 isolates characterized
- Of the 150 isolates from Chardonnay, 12 were *S. uvarum* unique to the winery 
- 588 were *S. cerevisiae* 

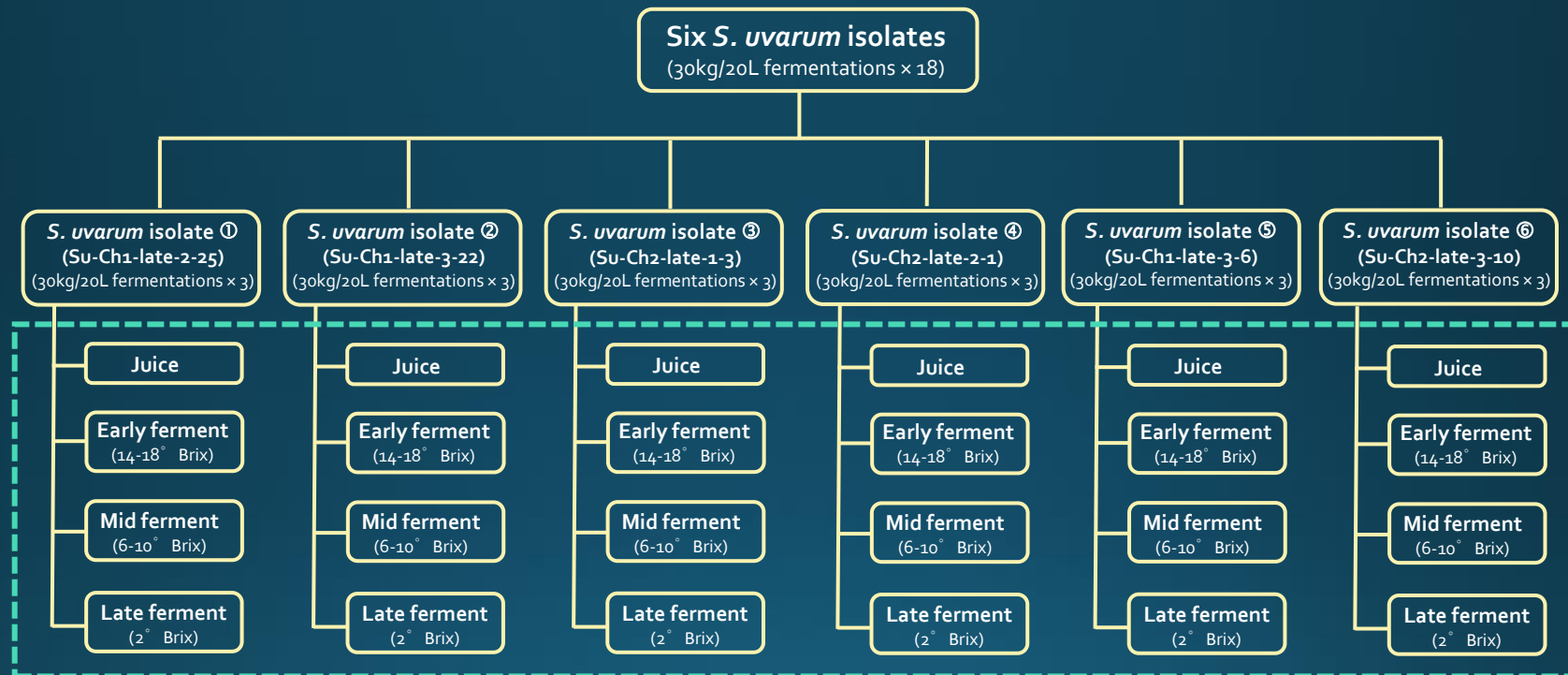
## Step 3: Test small scale ferments

### -6 isolates of *S. uvarum* retested



- All *S. uvarum* isolates were unique to the winery, the winery had never used the one commercial *S. uvarum* strain on the market (Velluto® BMV58 (Lallemand, Montreal, QC, Canada)).
- Tested 6 of 12 isolates for implantation efficiency into fermentations (Cabernet franc juice)
- Yeast paste cultures were prepared from each yeast isolate by Escarpment Laboratories (Guelph) to use in fermentations
  - No active dried culture process available for new yeast isolates
  - Escarpment Labs routinely prepares cultures for the brewing industry
  - Service accessible to wineries for culture preparation

# Experimental Schematic Diagram for *S. uvarum* Fermentation Trial



- There are six fermentation treatments in total and each treatment will be performed in Cab franc juice in triplicate.
- All fermentations are daily monitored for sugar consumption and ethanol via Foss.
- Samples will be taken for yeast analysis once the sugar falls into the above-specified ranges.

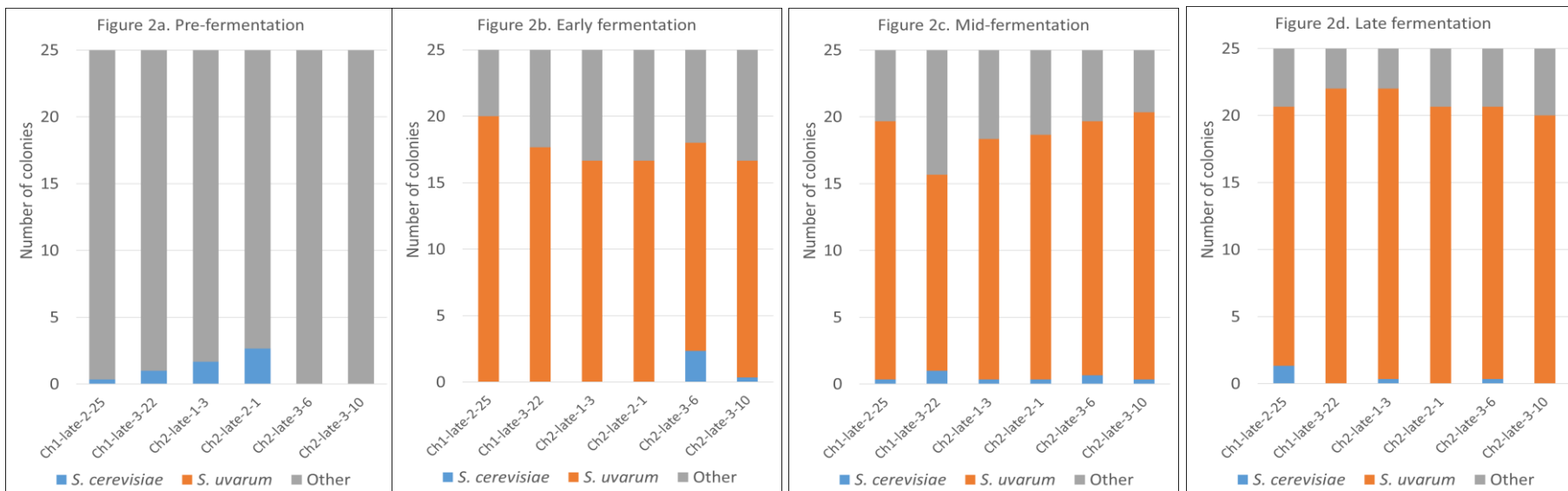


# Microbial analysis of time-course samples from Cabernet Franc fermentations

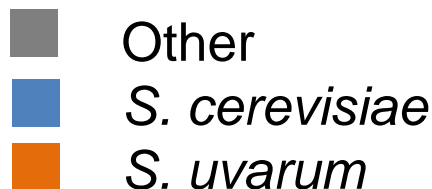


- 6 yeast isolates were used for inoculation into cabernet franc
- inoculated with *S. uvarum* paste cultures prepared by Escarpment Labs
- Samples from triplicate fermentations were plated on YPD with antibiotics to kill any bacteria then 25 colonies from each YPD plate were randomly selected and sub-plated on WLN plates
- Colonies were classified as *S. cerevisiae*, *S. uvarum* or other species on WLN plates, counted and the average colony numbers from the triplicate fermentation samples are shown in Figure 2.

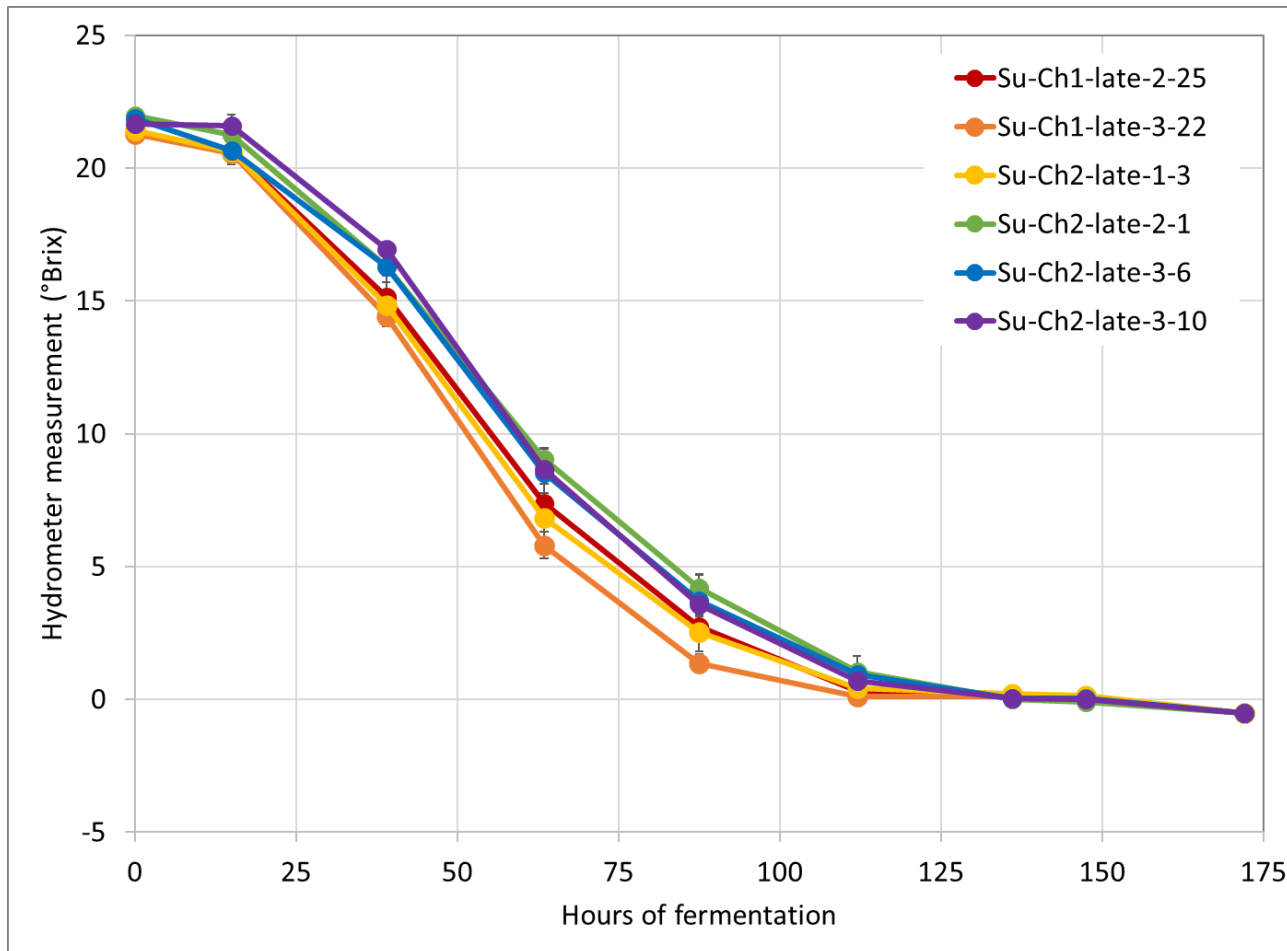
# Implantation of *S. uvarum* isolates into Cabernet franc fermentations



**Figure 2. Microbial analysis of time-course samples from Cabernet franc fermentations inoculated with *S. uvarum* paste cultures.**



# Fermentation performance



- All isolates fermented to dryness
- all allowed spontaneous MLF to proceed
- no faulted wines
- formal sensory evaluation of wines not possible through COVID but the winemaker happy with informal tasting and will proceed with commercial trials



# Strain identification by microsatellite analysis showed 3 different strains of *S. uvarum* from the 6 isolates



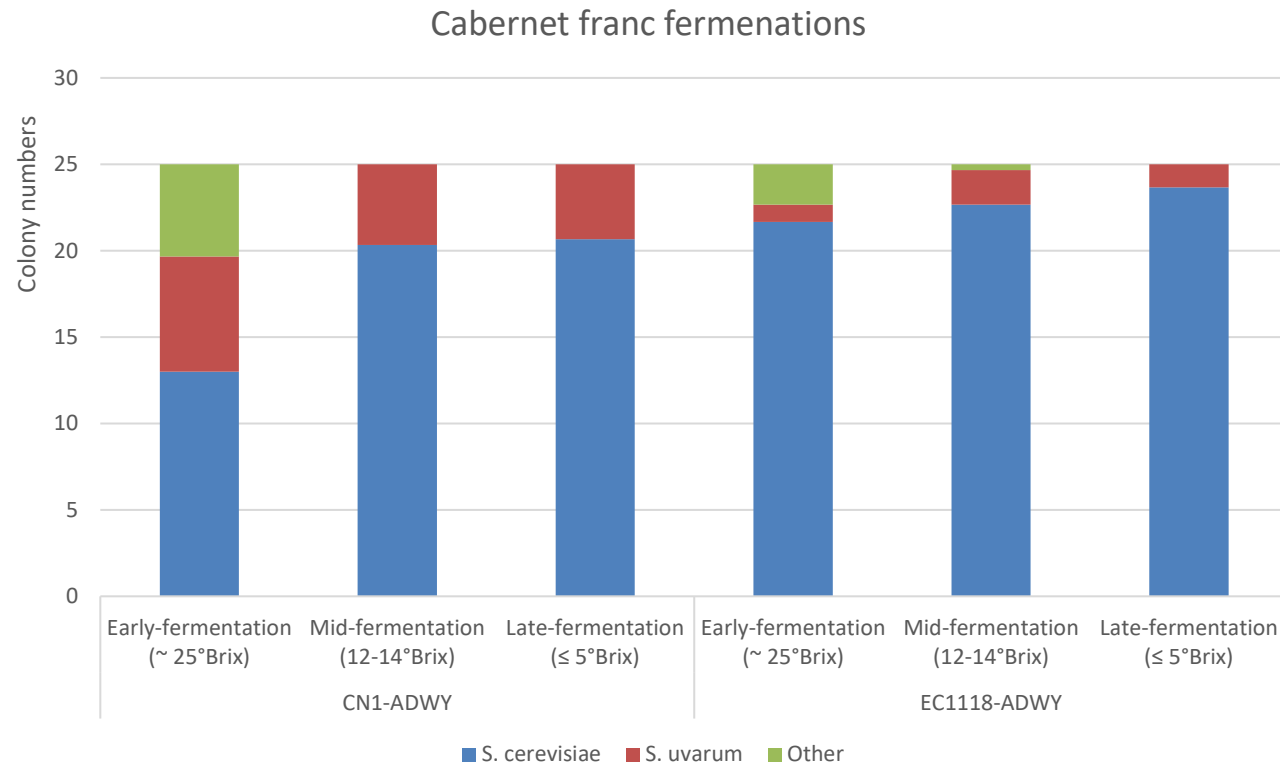
Yeast isolate code	Carboy code	Yeast strain type
Su-Ch1-late-2-25	1A	Strain A
	1B	
	1C	
Su-Ch1-late-3-22	2A	Strain B
	2B	
	2C	
Su-Ch2-late-1-3	3A	Strain C
	3B	
	3C	
Su-Ch2-late-2-1	4A	
	4B	
	4C	
Su-Ch2-late-3-6	5A	
	5B	
	5C	
Su-Ch2-late-3-10	6A	
	6B	
	6C	

## Step 4: Commercial fermentation



- Plan to trial *S. uvarum* isolates from this project at the commercial winery this fall
- We have successfully trialed another *S. uvarum* isolate we have been characterizing for a few years in fall 2021 - CN1
- That yeast was prepared by Lallemend as an active dry culture and performance compared to EC1118
  - Previous work had shown CN1 *S. uvarum* Shifted the sensory profile of Cabernet franc appassimento wine towards increased black fruit flavour and aroma
  - Reduced sourness and astringency vs. *S. cerevisiae* EC1118 commercial yeast

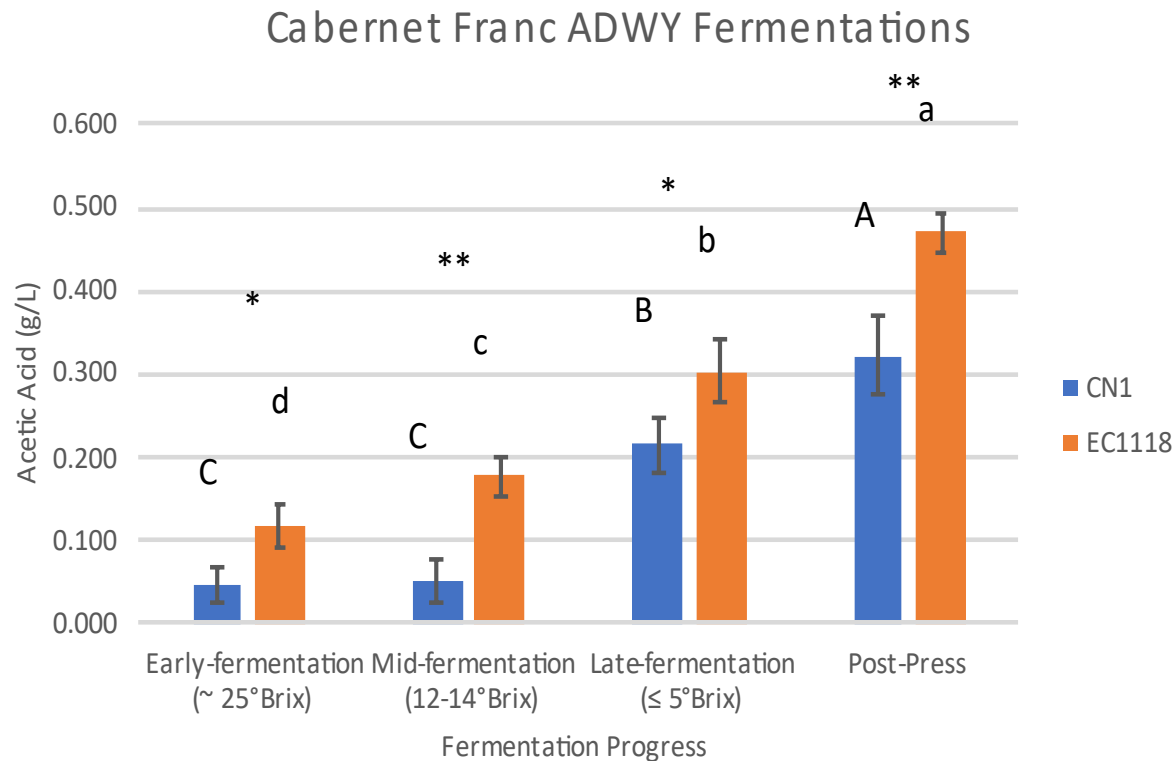
# *S. uvarum* CN1 implanted into the ferment and competed with *S. cerevisiae*



Juice had a substantial background of *S. cerevisiae* due to pre-fermentation processing of grapes but *S. uvarum* still established itself



# Commercial fermentation of ADY preparation of locally isolated *S. uvarum* CN1 fermenting Kiln dry fruit



- CN1 was successfully prepared in an active dry form, implanted and completed commercial fermentations of appassimento wine with significantly lower acetic acid production

# Summary



- Services are now available through CCOVI for winemakers to efficiently isolate their own natural yeast, store the cultures and have them prepared in a paste format through Escarpment Labs for use in commercial ferments as opposed to an active dry format
- Yeast isolated from a vineyard/winery operation may allow the winery to differentiate their wines in the marketplace by taking advantage of yeast biodiversity
- Natural yeast isolates are one more tool available to the winemaker, and sometimes these yeast have unique positive properties not present in commercial selections (e.g. CN1)

# Acknowledgements



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- Lallemant
- Escarpment Laboratories

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# Thank you!



## Questions?

## Cheers!

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